# Determinación de residuos de pesticidas en miel mediante la solución automatizada QuEChERS

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## Aplicación 053




## RESUMEN

El método de extracción QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) se desarrolló para la determinación de residuos de pesticidas en producción agrícola. SI bien el procedimiento QuEChERS se desarrolló para plantas, la técnica puede adaptarse a muchas otras matrices como miel.

El sistema robotizado AutoMateQ40 implanta el método QuEChERS efectuando; Adición de acetonitrilo (ACN) adición de sales tampón, agitando, mezclando, centrifugando la muestra, transfiriéndola aun tubo de limpieza (d-SPE) y midiendo el extracto final.

La presente aplicación valida la funcionalidad de la extracción mediante el AutoMate-Q40 para el control de neonicotinoides y otros pesticidas presentes en miel. Los compuestos fueron identificados y cuantificados mediante la técnica de cromatografía líquida con detección de masas en tándem (LC-MS/MS).


## Introduction

Colony Collapse Disorder (CCD) is an occurrence in which worker bees from the honey bee colony unexpectedly disappears. During the winter of 2006-2007, beekeepers began to report high losses of 30-90% of their hives.[1] With these high losses many hives cannot sustain themselves without the worker bees. Many regulatory agencies have theories about the cause of the CCD, including pesticide poisoning through exposure to pesticides applied to crops or for in-hive insect or mite control.

Neonicotinoids are a relatively new class of insecticides that affect the central nervous system of insects, resulting in paralysis and death.[2] The European Commission has restricted the use of clothianidin, imidacloprid and thiamethoxam, but the US has not adopted any such restriction.

The QuEChERS extraction method is the most applicable method, since it offers good selectivity, and sensitivity when extracting pesticide residues in honey. The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40 for the extraction of neonicotinoids and other pesticides in honey. Liquid Chromatography coupled to triple-quadrupole mass spectrometry (LC-MS/MS) was employed for the detection of pesticides in honey. Quantification was based on matrix-matched calibration curves.

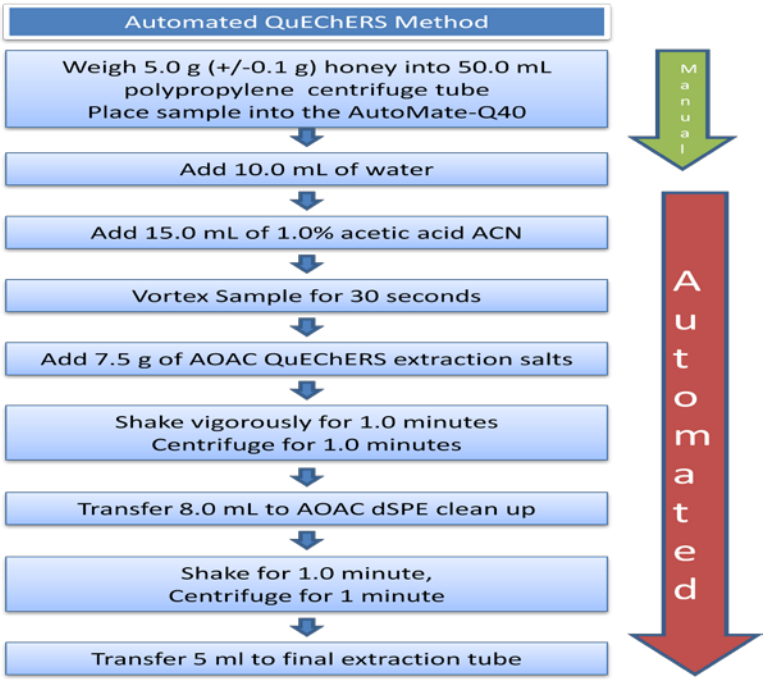
## Experimental Instrument Conditions

Honey was purchased from a local market in Ohio. The samples were prepared according to the procedure described in the “AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate”.[3] The sample was stored at room temperature until extraction.

Figure 1 shows the sample preparation and steps followed to extract pesticide residues from honey. For this analysis, the AutoMate-Q40 used 7.5 g of AOAC QuEChERS extraction salts ($MgSO_4$ and NaOAc).

The AutoMate-Q40 also used the AOAC version of $MgSO_4$ (1200.0 mg), PSA (400.0 mg) and C18 (400.0 mg) for the dSPE cleanup step.

**Figure 1** AutoMate-Q40 Extraction Parameter




Sample analysis was conducted using a Shimadzu Nexera HPLC system coupled to an AB SCIEX 4500 QTrap tandem mass spectrometer (MS/MS) via electrospray ionization (ESI). For separation of the compounds of interest, a Phenomenex Kinetex 2.6 um Biphenyl (50 x 2.1 mm) column was used. The unique biphenyl stationary phase combined with the high performance Kinetex core-shell platform provided exemplary chromatographic separation of the neonicotinoids by focusing on hydrophobic, aromatic, and polar-basic differences. Table I and Table II contain the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 2 shows the scheduled MRM chromatogram spiked at 400.0 µL/L.

| **Table I LC-MS/MS SRM Transitions and Parameters for AB SCIEX 4500 QTrap** | |
|---|---|
| Curtain Gas (CUR) | 20 |
| Ion Spray Voltage (IS) | 4000 |

| | |
|---|---|
| Temperature (TEM) | 450 |
| Collision Gas (CAD) | High |

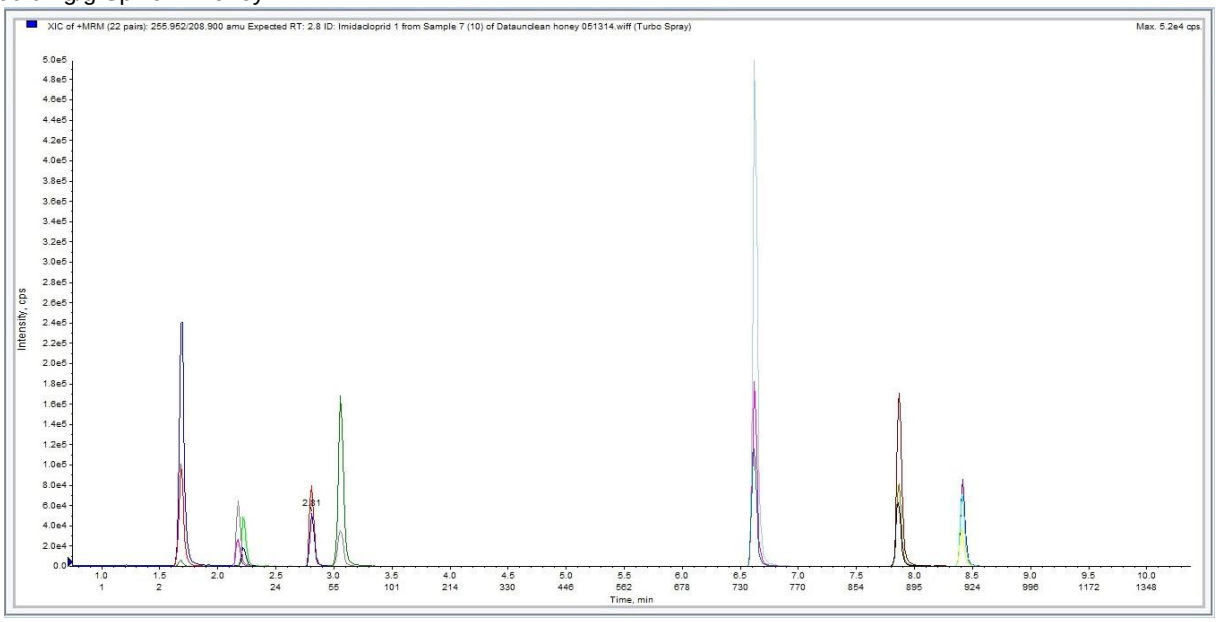
Analyte Transitions

| Compounds | RT (min) | Precursor Ion (m/z) | Quantization product Ion (m/z) | DP(V) | CE(V) | CXP(V) |
|---|---|---|---|---|---|---|
| Acetamiprid | 3.03 | 222.93 | 126.00 | 41 | 29 | 10 |
| Azoxystrobin | 6.60 | 404.04 | 372.10 | 66 | 23 | 12 |
| Chlorpyrifos | 8.40 | 349.86 | 197.70 | 56 | 23 | 14 |
| Clothianidin | 2.20 | 249.91 | 168.80 | 21 | 17 | 20 |
| Coumaphos | 7.85 | 362.93 | 226.80 | 91 | 33 | 24 |
| Imidacloprid | 2.79 | 255.95 | 208.90 | 46 | 17 | 30 |
| Nitenpyram | 1.65 | 270.99 | 224.90 | 35 | 15 | 14 |
| Thiamethoxam | 2.15 | 291.94 | 210.90 | 36 | 15 | 16 |

**Table II Shimadzu Nexera LC Parameters**

| | | |
|---|---|---|
| Column | Kinetex 2.6 um Biphenyl | |
| Dimensions | 50 X 2.1 mm | |
| Mobile Phase | A:0.1% Formic Acid in Water | |
| | B:0.1% Formic Acid in Acetonitrile | |
| Gradient | Time | %B |
| | 0.10 | 5% |
| | 10.0 | 70% |
| | 12.0 | 70% |
| | 12.1 | 5% |
| | 14.1 | Stop |
| Flow Rate (mL/min) | 0.5 | |
| Column Temperature (°C) | 40 | |

**Figure 2** 400.0 ng/g Spike in Honey



## Experimental Results

Automating the QuEChERS extraction enables a fast, easy, reliable and more reproducible extraction. The AutoMate-Q40 offers significant labor savings, while improving the reproducibility and consistency between samples.

A precision and accuracy study was performed using the AutoMate-Q40. A 2.0 µg/mL stock pesticide solution was used to fortify the honey samples. Check standards were fortified at 10.0 and 30.0 µg/L using the AutoMate-Q40's ability to make standard additions. This translates to 30.0 and 90.0 ng/g for honey. These QC samples were quantitated against their corresponding matrix matched calibration curve.

The analysis was performed in two parts. The AutoMate-Q40, can extract the honey samples with and without a dSPE cleanup. Two sets of data will be presented in this precision and accuracy study. Figure 3 and Figure 4 show data using the AutoMate-Q40 to extract unclean honey samples. Figure 5 and Figure 6 show data that used the dSPE cleanup option on the AutoMate-Q40.

**Figure 3** Average Recovery for Unclean Honey Samples

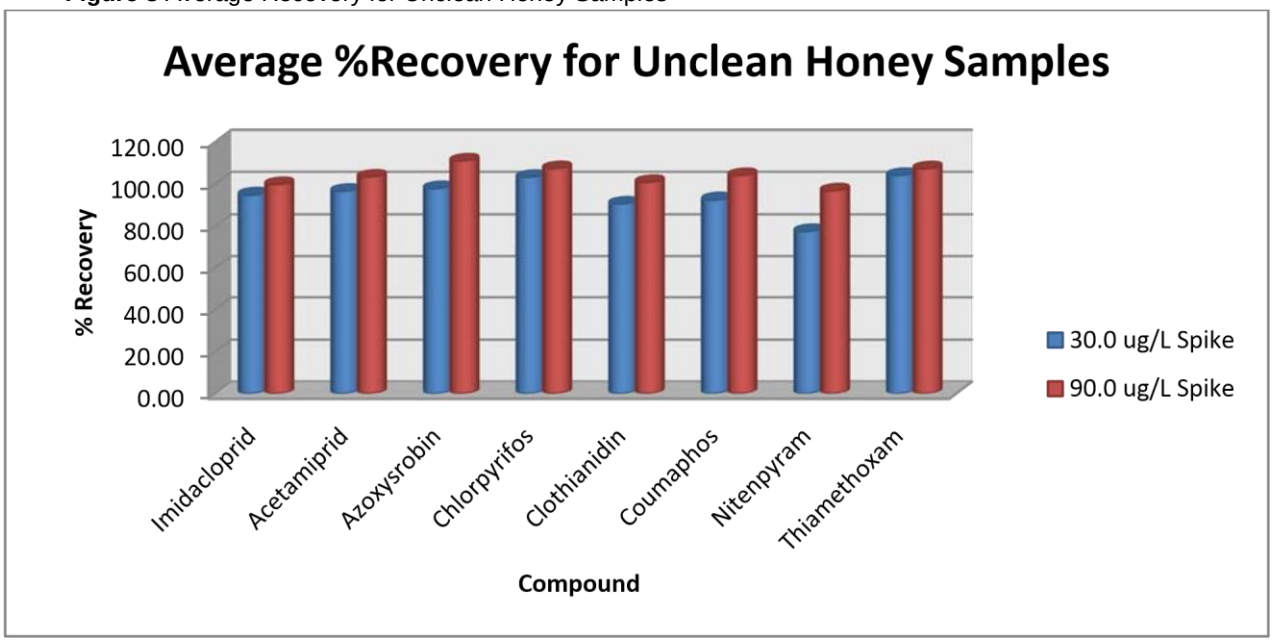



**Figure 4** Average %RSD for Unclean Honey Samples

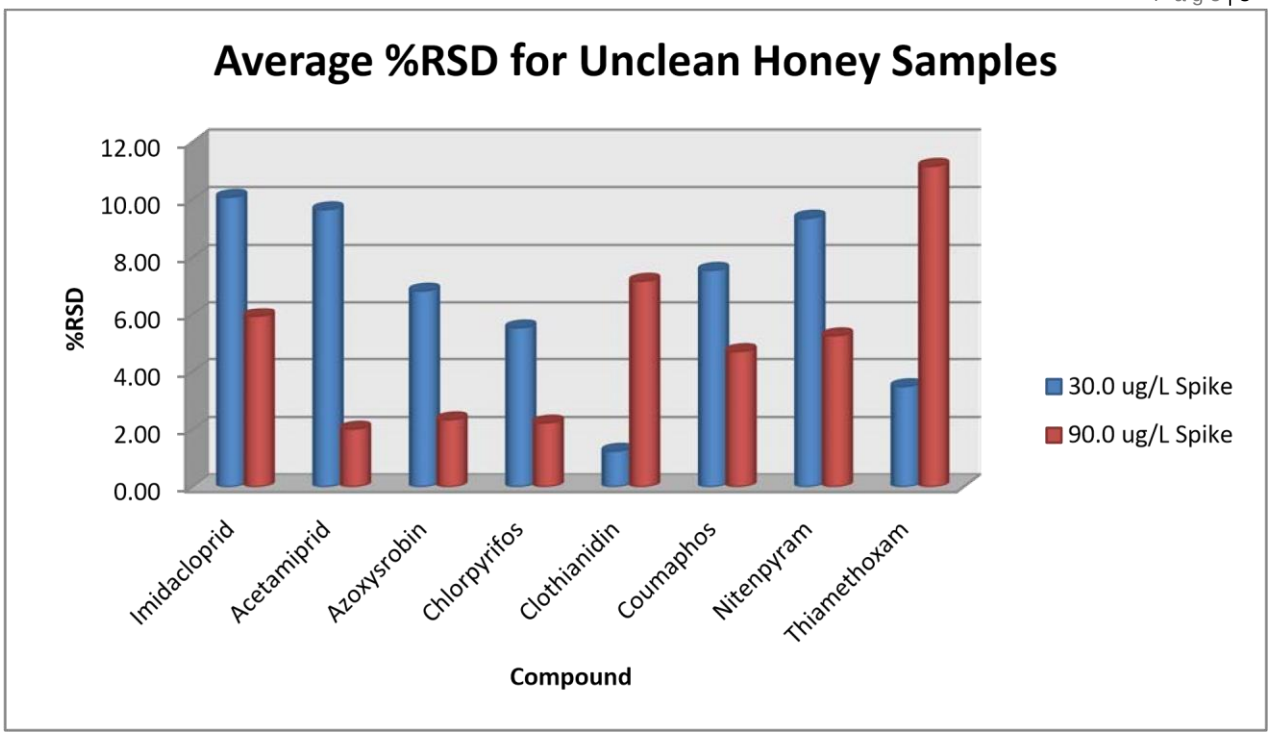



**Figure 5** Average % Recovery for Clean Honey Samples

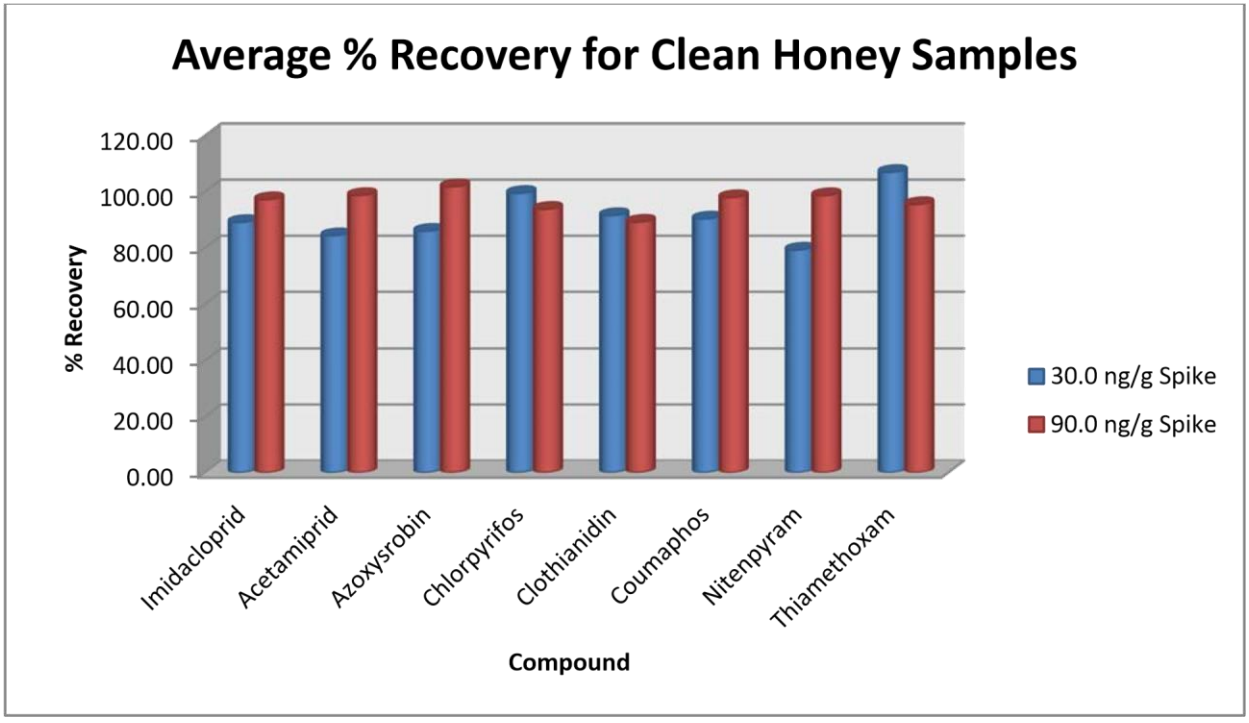
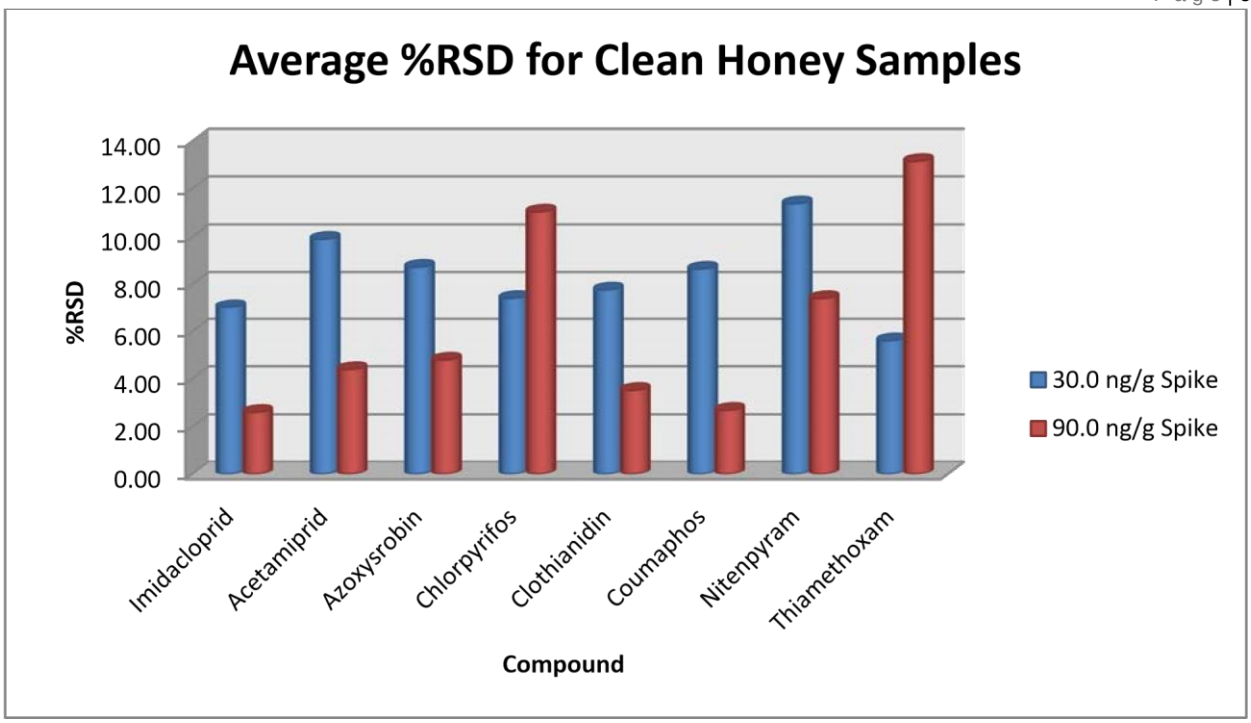



**Figure 6** Average %RSD for Clean Honey Samples




Figures 4-6 show that data derived from AutoMate-Q40 extracted pesticide residues in honey exhibited recoveries ranging from 77.55% to 107.25% for all spiked QC samples. The AutoMate-Q40 also demonstrated great precision; ranging from 1.25% to 13.11%RSD for the spiked QC samples. These spike recoveries fall within the recommended mean values for the Document N˚ Sanco/12495/2011[4]. This document states that the mean recoveries must fall between 70% to 120% with a RSD <20%.

## Conclusion

This study demonstrates the Automate-Q40's ability to successfully process honey samples for pesticide residue by the QuEChERS extraction method. By automating the liquid handing, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the AutoMate-Q40 frees the scientist from a labor-intensive extraction method and exposure to unhealthy chemicals. The extraction process is faster, more reliable, and easier. This enables time and labor savings, while improving consistency and reproducibility of the extraction. As shown above in Figures 4-6 the combined pesticide spikes recoveries of 93.74%, with an average RSD of 5.92% exceed the requirement outlined in The Document N˚ Sanco/12495/2011. These numbers indicate superb precision and accuracy thus validating the performance of the AutoMate-Q40 to adequately perform the QuEChERS pesticide extraction method for honey.

## References

1. http://www.epa.gov/pesticides/about/intheworks/honeybee.htm
2. Galeano K.; Scordino M.; Sabatino L.; UHPLC/MS-MS Analysis of Six Neonicotinoids in Honey by Modified QuEChERS: Method Development, Validation, and Uncertainty Measurement
3. AOCA Official Method 2007.07 Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry, First Action 2007
4. Method Validation and Quality Control Procedure for Pesticide Residues Analysis in Food and Feed (Document N˚ SANCO/12495/2011)

## Acknowledgements

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